INTRODUCTION

Histology is a term ***derived*** from the Greek words ***"Histos"*** meaning tissue and ***"Logia"*** meaning We could think that Histology refers to the study of only tissues, but this is not so As you know Anatomy could be divided into that which is visible to the naked eye -cross Anatomy- and that which is seen only with the aid of a microscope -microscopic Anatomy-and this is our subject. **It** can be further classified into:

a- Cytology (study of cells);

c- Organology (study of organs).

AS the' *term "Histology"* was introduced by Bichat (18O2) for different ***groups*** of cells, it remains as the study of tissues *and* also covers all microscopic anatomy . Thus,***Histology***refers to study of cell, tissues and organ systems. it embraces the study of function as well the structure , and also provides the structure basis for the study of physiology . it is necessary a knowledge of the normal in order to study abnormal -Pathology- that deals with alterations of the structure and function of the body, its organs, tissues, and cells.

It could be stated that Histology was born when the microscope was developed. To study histology there are two important considerations to hear in mind:

1- The kind of microscope used, and

2- The preparation of the tissue

MICROSCOPY

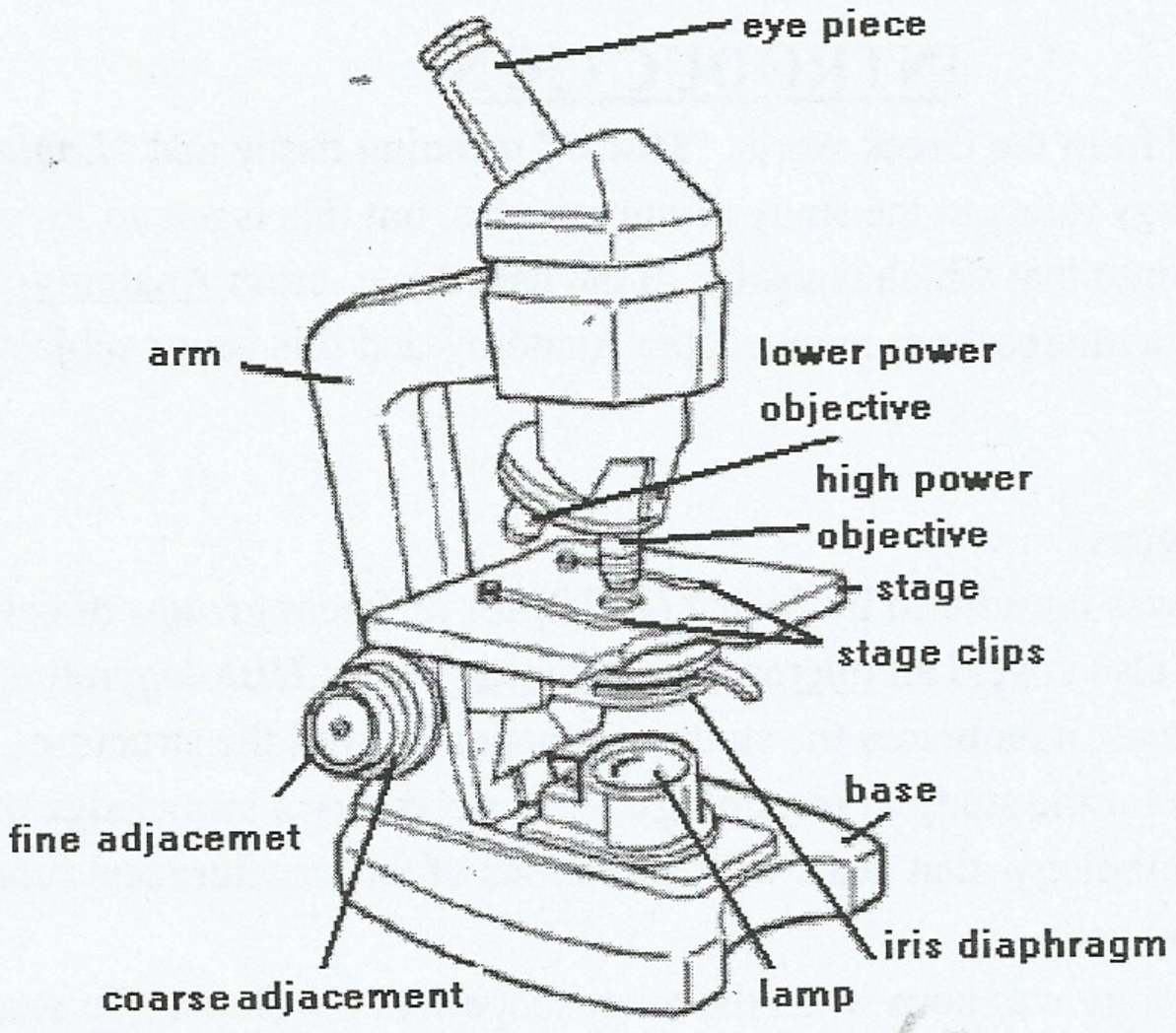
There are many types of microscopes. They are classified according to the type of light source used. in most general use is the optical microscope which uses visible light and is known as OM or LM. We will refer mainly to the LM and to the electron microscope –EM . which uses as illumination source a beam of high velocity electrons accelerated in a vacuum. There are many other types of microscope as you can see in any textbook. Now we will stop for a minute to have some facts cleared about the LM and the EM.

It is called "**resolution**" the "resolving power" of any microscope, that is. The capacity to clearly separate twopoints.

MTCROSCOPE	Resolution	MAGNIFICATION
Light	0.2μ	1500X
Electron	0.2mu	300,000 X

Recently it has been approved to replace those units by units related to the metric system. Since students will read both terminologies in books, they should be acquainted with both of them.

OLD TERMINOLOGY	NEW TERMINOLOGY
Micron (μ.)	Micrometer (μm)
Millmicron (mμ)	Nanometre (nm)
Amstrong unit (A°)	0.1 nm



Compound Microscope used for specialized study of the cells Include dark field microscope, phase contrast microscope and fluorescence microscope. Very minute subcellular structures can be studied with the help of electron microscope (TRANSMISSION and SCANNING)

The main instrument usedin the study of histology is bright field light microscope also called light microscope. Microscope presents an enlarged image of the specimen. Tissues and cells which cannot be seen with naked eye can be seen with the help of the microscope. The main components of a microscope are as follows:

A- OPTICAL PARTS:

1- Light source: Light is provided through a 6 volt 20 watt tungsten bulb. Bulbs with brighter illumination are used in advanced research microscopes. Reflected light from sun or tube light using reflecting mirror is still used but is not satisfactory.

2- Condenser: The parallel light rays emerging from the light source are converged onto the object with me help of a lens called condenser.

3- condenser: Light passes through the hole and illuminates the object under study.

4- Objective: Objective is a compound lens and is made of series of convex and concave lenses. It enlarges the image of the object and transmits to the eyepiece. The generally used magnifications of objective are 10x, 4Ox and 1OOx.

5- Eyepiece The eyepiece lens gathers the image transmitted from the objective and relays to the eye of the observer. It also further magnifies the image. Microscope is monocular if only one eyepiece is present and binocular if two eyepieces are attached for use for both the eyes simultaneously.

B- MECHANICAL PARTS

1- Mechanical stage: A rectangular stage with a large hole in the center and clips to grip the object or glass slide is placed above the condenser: Light passes through the hole and illuminates the object under study. 2. Arm. 3. Base. 4. revolver 5. coarse and fine adjustment6. draw (ocular) tube.
7. roak and pinion.

PREPARATION OF TISSUE:

Preparation of Tissues have to be suitable prepared for microscopic examination. There are two methods:

1. Direct observation of living cells.
2. Fixed or preserved dead cells.

Living tissues are usually more difficult to handle and are only available for a short period of time. In our practices, fixed and stained preparations of tissues, which are permanent, will be used.

PARAFFIN METHOD:

1.**Fixation:** A piece of tissue cut out of an animal and left without treatment will soon die and dry up. Even if it is kept wet, the cell enzymes will digest it. This process is called autolysis (self dissolution). It will also be susceptible for bacterial and fungal attack, a process called putrefaction. **Fixation** is therefore needed to prevent autolysis and putrefaction. It is also needed to make the tissue resistant to subsequent treatments, e.g. embedding, and to make it colorable by suitable dyes. The following is a list of chemicals used as fixatives, either alone or mixed in specific proportions. Formalin (10 % solution of the formaldehyde gas in water for 24 hours) SUZA,ZINKER and bouins

2 — Wash in running tap water for several hours. Three or four hours are enough if formalin is the fixative used.

3 — **Dehydrate**, (i.e. remove water or **Dehydration**) in ascending grades of alcohols (70 %, 90 %, 96 % then 100 %). The time of dehydration depends on the size of the piece of tissue. For a piece 5 mm thick 2 hrs in each change of alcohol will be enough. to prevent tissue shrinkages

4 — Alcohol is then removed by immersing in xylol or benzene. This step is called **Clearing**, since the tissue becomes translucent. 1 hr is sufficient for clearing.

5 — The tissue is **impregnated** with two or three changes of molten paraffin then embedded in it in the form of a block. The time of impregnation is 2 - 3 hours.

6 — **Sections** are cut from the paraffin block using an instrument called the **Microtome**. The ideal thickness of sections is between 8 and 10 microns (μ).

7 — **Mounting**: Sections are spread by floating them over warm water, (40 - 45 °C). Each section is picked up by a clean glass slide coated with a very thin film of egg albumin. The slides are then put in a warm (40 °C) oven and left to dry overnight. As the sections dry, they are stuck to the slides by the albumin film.

8___ **Staining** the most used stain is combination of Haematoxylin (H) and eosin (E).

Result: Nuclei are stained blue with haematoxylin while the cytoplasm is stained red with eosin. Generally speaking, any basophilic structure in the cell will stain with haematoxylin and any acidophilic structure will stain with eosin.

9- **Mounting**

Definition: a basophilic structure is one which has the affinity for basic dyes because of its acidic nature. For e.g. the nucleus is rich with an acid known as DNA (deoxyribonucleic acid) and therefore is basophilic. The acidophilic structure, on the other hand, is one which stairs with acidic dyes because it contains a base (like a basic protein) as in the case of the cytoplasm of some cells.

All the student-sections are stained with H & E unless otherwise stated

THE CELL

*Definition: It is structural and functional unit of all living tissues (Plants and animals). Cells have different shapes and size.
*Size of the cell: Lymphocyte is one of the smallest cells (6um) While fat and ova are the largest cells (160 um).
*Shape of cell: some cells rounded, other oval, flat, cubical or columnar.

Composition:

1- Major component of cell is water(75%)
2- Numerous inorganic components:
a- Chief intracellular cation is K+.
b- Chief intracellular anions are HCO3-, HPO4-2, SO4-2.
3- Macromolecules suspended in the basic aqueous soup: Nucleic acids ,DNA and RNA
4- Proteins :Made up of amino acids. May be conjugated with lipids, sugars or nucleic acids.
5- Lipids :May be conjugated with other macromolecules.Important constituent of membranes.
6- Carbohydrates: Polysaccharides,Polysaccharide-protein complexes.Glycoproteins.Glycolipids.

The **protoplasm** is subdivided into two compartments in the body cells, the cytoplasm and the nucleus. The cytoplasm is a “colloidal solution" and within it are the cytoplasmic bodies, “organelles” which are living structural components, and “inclusions” which are non-living accumulations. The nucleus contains the genetic information necessary for many cellular activities, the karyoplasms and the nucleolus.

Properties of protoplasm:.

The protoplasm properties indicate the functions of the cells, and some are more developed by particular type of cells. They are the following:
1. IRRITABILITY is the capacity to respond to a stimulus and is the expression of life itself
2. CONDUCTIVTTV is the property to transmit a wave of excitation. It is developed at nerve and muscle cells.
3-CONTRACTILITY is the property of Changing shape. it is highly developed in muscle cells.
4. RESPIRATION: is the process whereby cells interact with nutrients and oxygen to produce energy, waste products, carbon dioxide and water.
5.ABSORBTION: is the capacity that the cell has to let get in substances from the environment.
6. SECRETION is theprocess by which a cell delivers useful material externally.
7. EXCRETION is the elimination from the cell of waste products of metabolism.
C. GROWTH is the increase in the size of cells as a result from the increase in the amount of protoplasm. There is a maximum size for each cell type Beyond themaximum size, usually cell division occurs.

Structural Organization

Cytoplasm

Cytoplasmic matrix(Cytosol, Ground Substance). Basic structureless component of the cytoplasm. Consists of large molecules of protein, soluble enzymes, mineral salts, and other absorbed soluble substances.

Organelles and inclusions suspended in the cytoplasmic matrix.

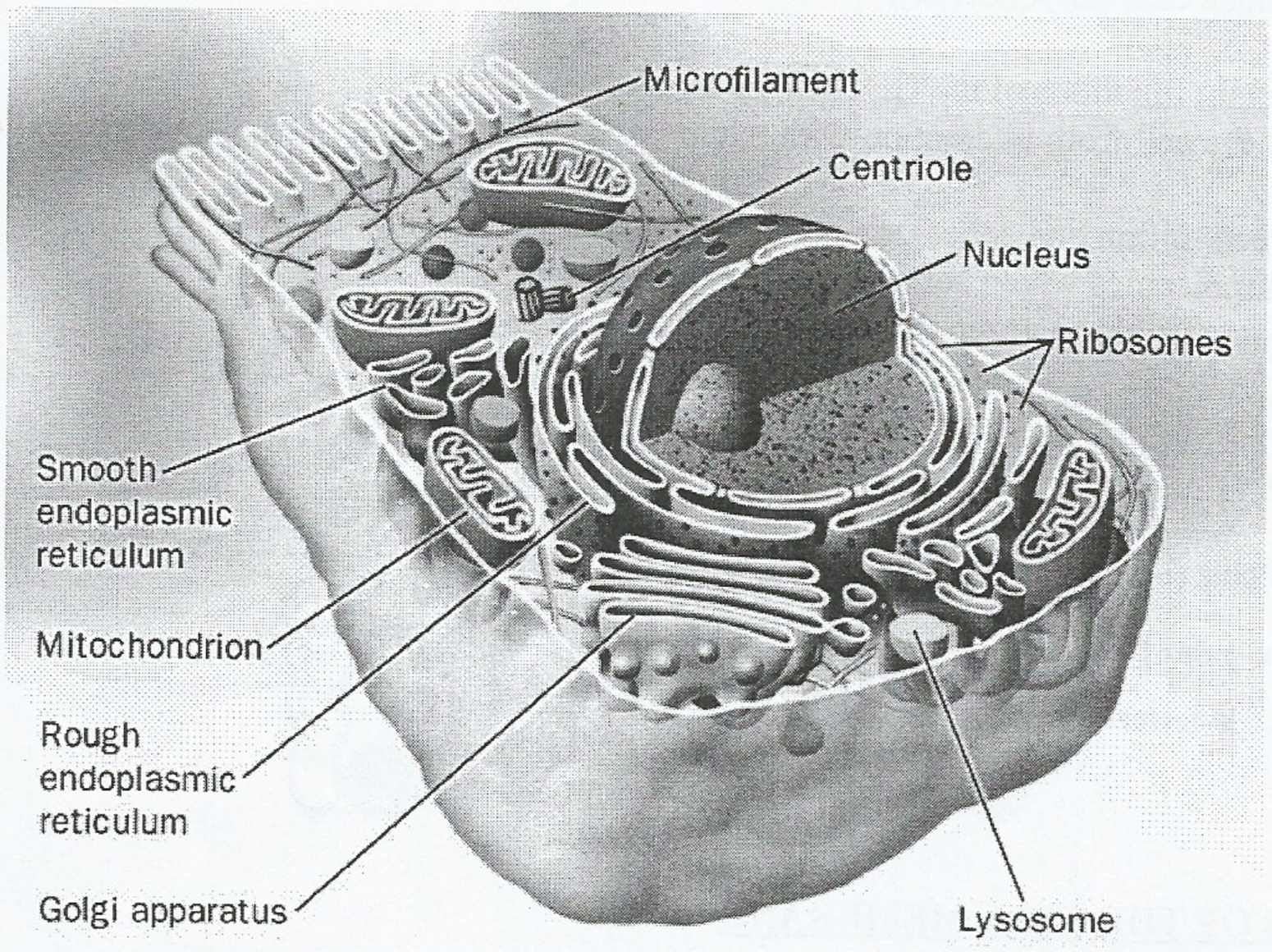
Cytoplasmic Organelles: they are differentiated structures, essential for vital processes of the cell(respiration, digestion, secretion, excretion).

Inclusions: they are not essential for vitality of cells. They may be present **Cytoplasmic Organelles** :They are membranous and non-membranous

Cytoplasmic Organelles

A- Membranous Organelles:

1- Cell membrane (plasma membrane)
2- Mitochondria.
3- Endoplasmic reticulum (rough & smooth)
4- Golgi apparatus (Golgi body or Golgi complex)
5- Lysosomes
6- Peroxisomes(microbodies)



B – NON- Membranous Organelles:

1- Ribosomes.
2- Centrioles
3- Cytoskeleton(Microtubules And Filaments Structures).

A- Membranous Organelles:

1-Cell membrane(plasma membrane, plasmalemma):

Is very difficult to be seen by light microscope. It is an ultrathin membrane that surrounds the cell.
By E.M: appears as two dark layers, separated by a light one, (trilamellar membrane or unit membrane)
The molecular biology of the cell membrane: is composed of lipid bilayer associated with proteins and carbohydrates, giving together a mosaic appearance.

a) Phospholipids molecules arranged into 2 layers (bilipid layer). Each molecule has a polar & a non-polar end.

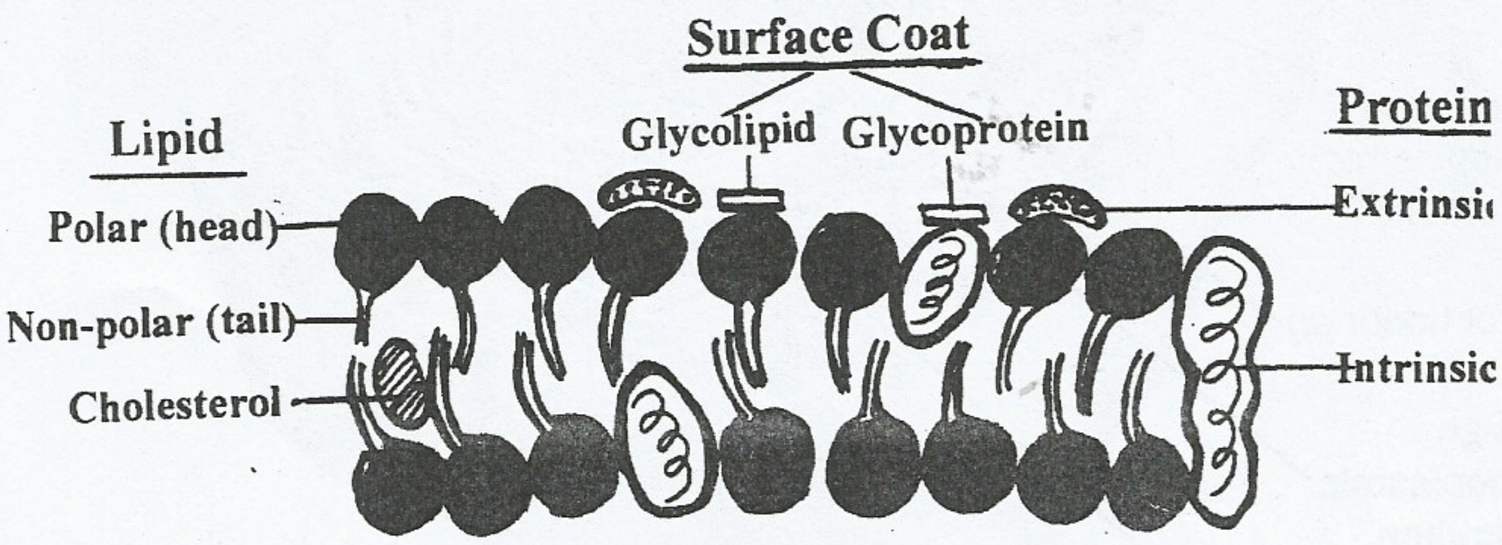
* The polar end (head) is hydrophilic and directed outwards, so the heads are found on the outer and the inner (Cytoplasmic) surfaces of the cell membrane.

* The non-polar end (tail) is hydrophobic and directed inwards where the tails of the two layers interact with each other forming a stable centre for the cell membrane.
b) Cholesterol molecules are incorporated in the hydrophobic regions of the cell membrane.

b) Protein Component: It consists of:
a) Extrinsic protein: It is formed of loosely attached protein molecules, found on both surfaces of the cell membrane.
b) Intrinsic protein: It consists of small or large protein molecules, either floating in the bilipid layer or fixed by certain cytoskeletal components.
c) Carbohydrate Component: It consists of short chains of polysaccharides conjugated with either proteins or lipids of the external surface of the cell membrane (glycoproteins and glycolipids), forming the surface coat (Glycocalyx).

Surface Coat:

The most important functions of the Glycocalyx are:
a- Protection of the cell from interaction with inappropriate proteins and also from chemical or physical injury.
b- Cell to cell recognition and adhesion.
c- Have certain molecules which act as specific receptor.
d- In RBC, it contains blood group antigens.



FUNCTIONS OF THE CELL MEMBRANE

Is a specific, selective process utilizing energy and comprises endocytosis and Exocytosis

Endocytosis includes:

A - Phagocytosis (cell eating).
B - Pinocytosis (cell drinking).
C - Receptor Mediated pinocytosis

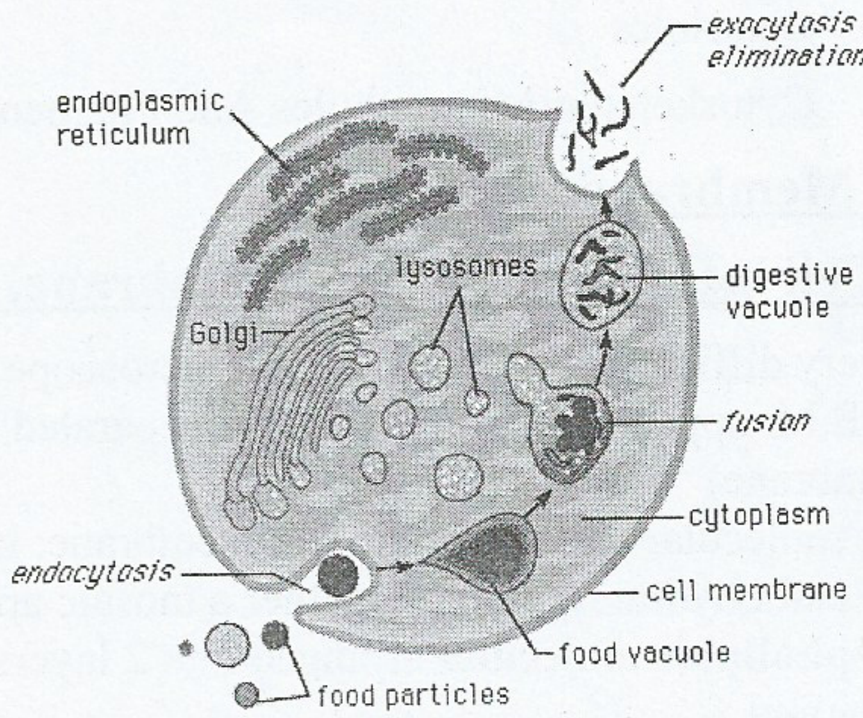
A- Phagocytosis:

It is the processes of intake of solid particles from the environment by extending processes that envelope and draws the particle into cytoplasm.

B- Pinocytosis:

The cell membrane invaginates to produce a deep, narrow pit.
The membrane near the surface of this pit then fuses, and small vacuole or pinocytic vesicle containing the extra- cellular fluid is pinched off and enters the cell.

C-Receptor- mediate endocytosis:



In receptor- mediate endocytosis, the interaction of the very specific molecules in the extracellular environment with specific membrane receptor proteins causes the membrane to invaginate, fuse, and pinch off to form a vesicle.

SPECILIZITIONS OF THE CELL MEMBRANE

1-Modification at the free surface of the cell includes microvillus, Cilia, sterocilli, and flagella.
2-Modification at the lateral surface of the cell includes junctional complexes at Epithelium tissue and membrane inter-digitations.
3- Modification at the basal surface of the cell includes basement membrane and basal in folding.

2- Endoplasmic reticulum

Flattened, rounded or tubular vesicles or cisternae anastomosing with one another, and continued frequently with the nuclear envelope.

Granular(rough) ERstudied with ribosomes, usually arranged in the form of flattened cisternae stacked in parallel, are abundant in secretory cells with protein synthesis..

A granular(smooth) ER lacks ribosomes, usually vesicles or tubules of membrane-bound, concerned with steroid synthesis. Drug detoxification occurs in hepatocyte. synthesis and storage of lipids and cholesterol. Muscle contraction and relaxation ,by acting as calcium pump

Golgi apparatus

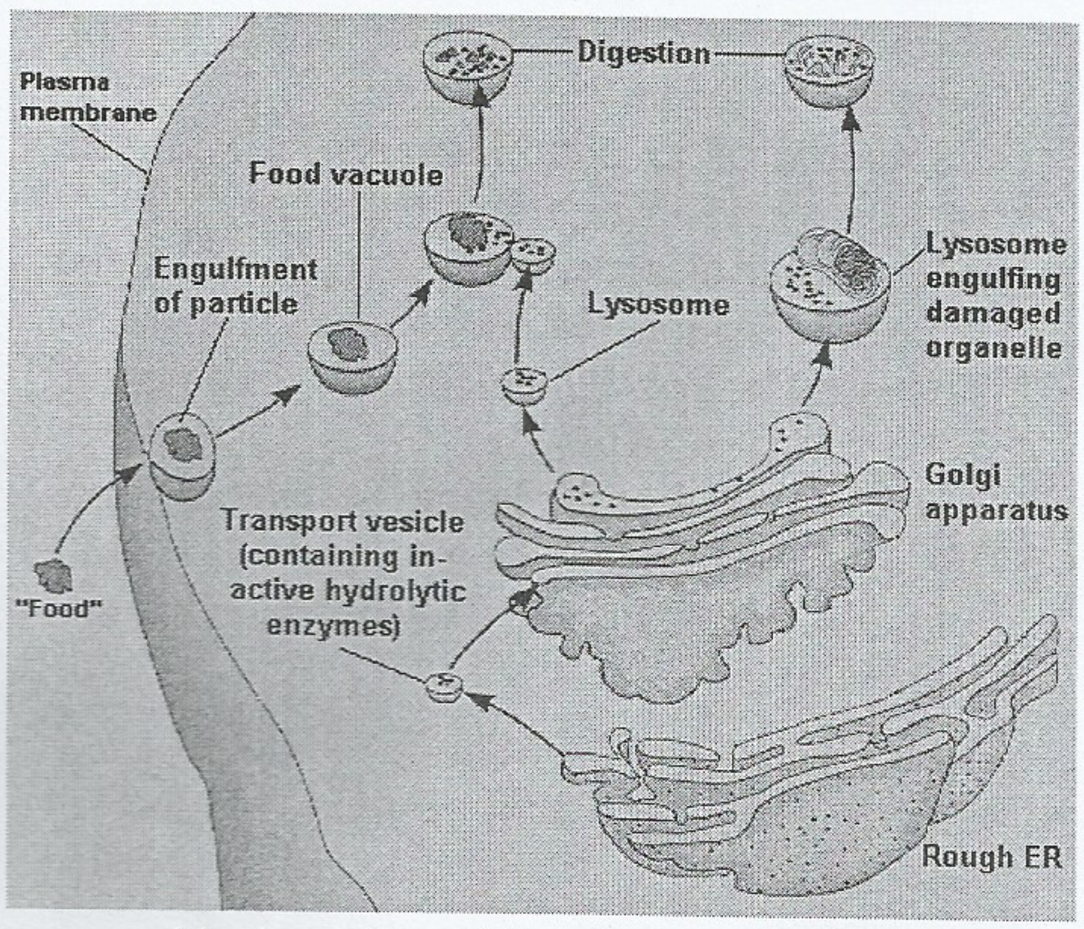
A group of piled up flat saccules or cisternae arranged in parallel array, located in juxtanuclear area. Convex surface (forming face), fenestrated, flattened cisternae with transfer vesicles derived from ER.

Concave surface (maturing face), dilated cisternae associated with condensing vacuoles or secretory granules.

- Active role in packing of protein-rich materials.
- Conjugation of sugars and proteins in glycoproteins

Lysosomes

Membrane-bound vesicles 0.2-0.5μ in diameter, containing hydrolytic enzymes.
Active intracellular digestion for ingested materials (Phagocytosis) or normal cellular organelles (autophagy)
Synthesized in RER, transferred and packed in Golgi apparatus as primary lysosomes containing inactive enzymes.
Secondary lysosomes, vacuolar structure engaged in current or past digestive activity. **TYPES:**
1-Autophagic lysosomes: formed by fusion of primary lysosomes with a dead organelle as mitochondria.
2- heterophagic vacuoles: formed by fusion of primary lysosomes phagocytic vesicles (phagosome) ,containing exogenous substance, bacteria.



3-multivesicular bodies: formed by fusion of primary lysosomes with pinocytic vesicles.
4-residual bodies: are secondary lysosomes which contain indigestible materials, that are discharged by exocytosis.

Mitochondria:

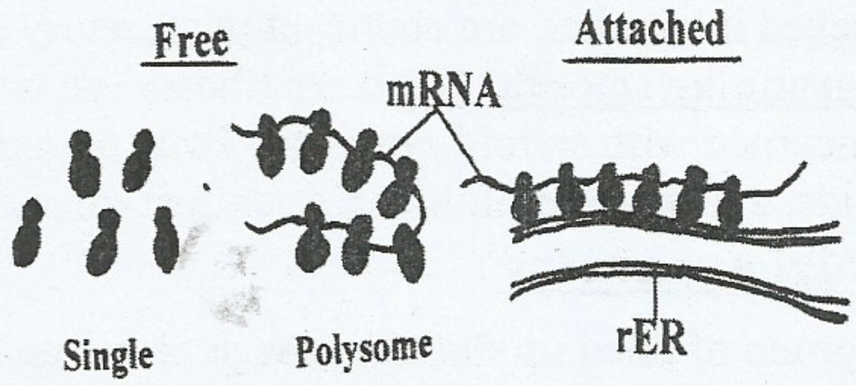
LM: They appear as rods, granules or filaments, after staining with Iron H or Janus green.
- They vary in number and size according to the function of the cell Mitochondria

EM:
- Each mitochondrion is surrounded by 2 membranes. The outer membrane is smooth, while the inner one forms incomplete and alternating shelves (cristae). Cristae increase the surface area for deposition of enzymes that function in oxidative phosphorylation.
- The cavity is filled with mitochondrial matrix, which contains:
  - Enzymes of Krebs cycle and fatty acids oxidation.
  - Phospho-lipo-proteins (matrix granules) that bind calcium (Ca) and magnesium (Mg).
  - Contains its own genetic apparatus (DNA and RNA).

Functions:
1- Cell respiration and production of adenosine triphosphate (ATP), the primary source of energy for the cell. That is why they are called the power-house of the cell.
2- They can form proteins for themselves and undergo self replication.

B – NON- Membranous Organelles

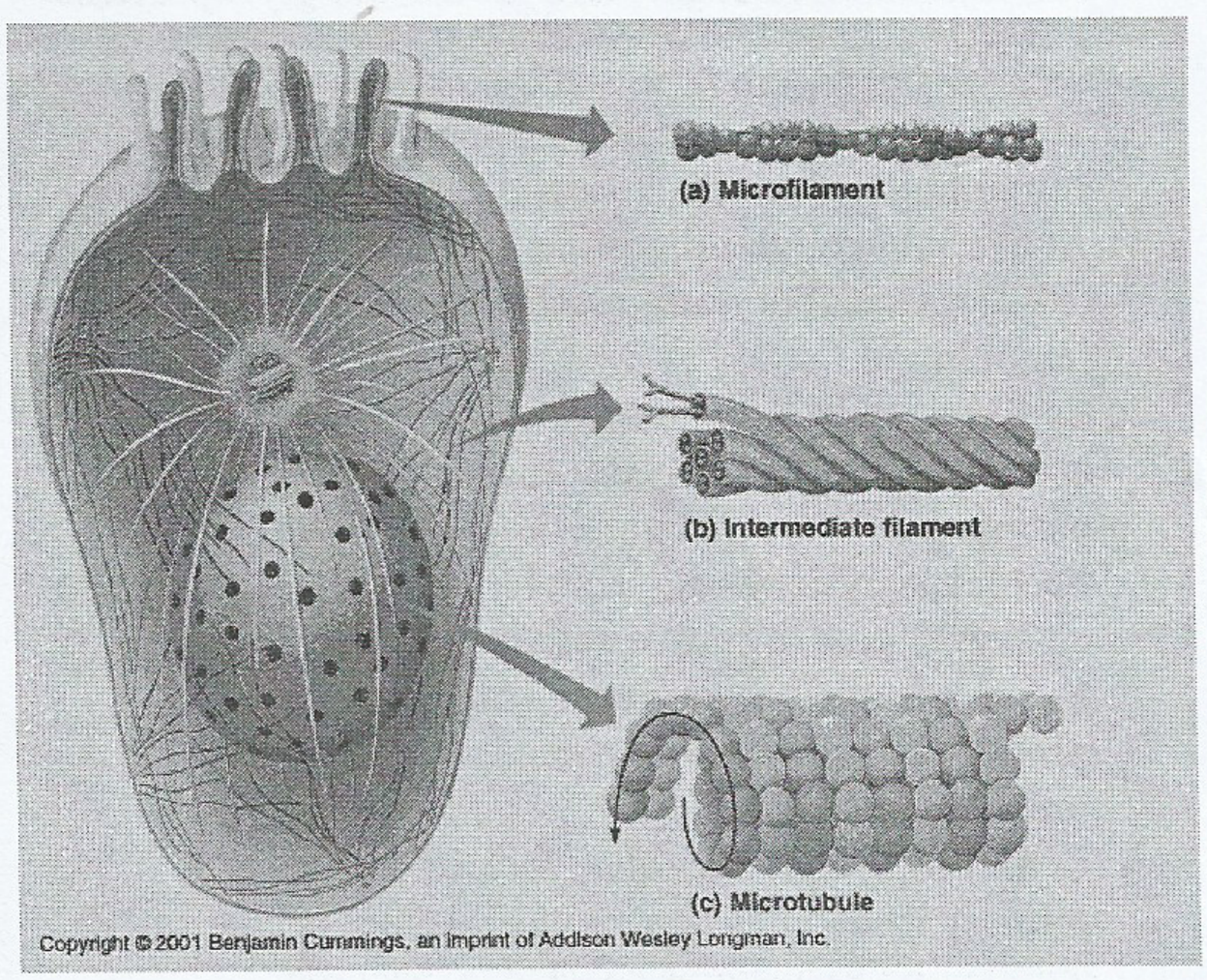
Ribosomes :



Small electron dense particles, 15-20nm in diameter.
RNA(60%) and protein(40%) responsible for the basophilia, known as basophilic body, ergastoplasm, Nissl body.
E/M: Appear as electron dense granules, which may be
— Free, either singly or in groups, connected together by a messenger RNA (mRNA), to form clusters or spiral chains (polyribosomes or polysomes).
— Attached, arranged on the membrane surface of the rough endoplasmic reticulum.
Free ribosomes synthesized the protein for intracellular use,
attached ribosomes associated with synthesis of protein for export.

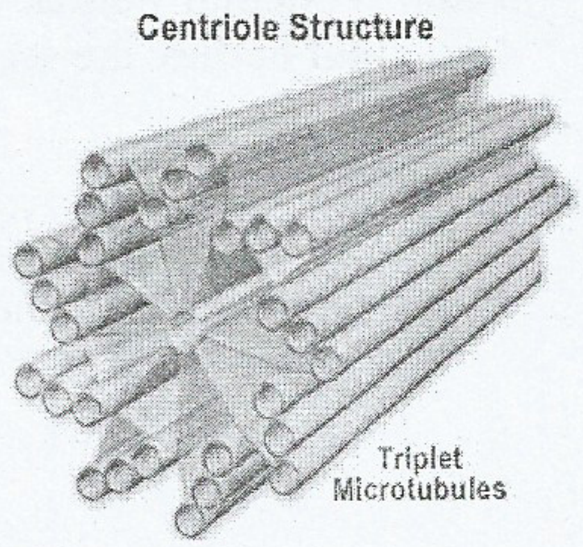
Microtubles

Rod- like or pipe-like structures 24nm in diameter with the wall 5nm thick, several micrometer long.
Composed of 13 protofilaments, which made up of protein subunit (tubulin dimers).
·Appear and disappear by polymerization and depolymerization of a pool of these subunit.
Growth blocked by colchicines (antimitotic alkaloid that bind tubulin).
Function: - Rigid nature - cytoskeleton, intracellular transport.
Provide the basis for Centrioles, cilia and mitotic spindle fibers.



Centrioles (cell center, centrospheres)

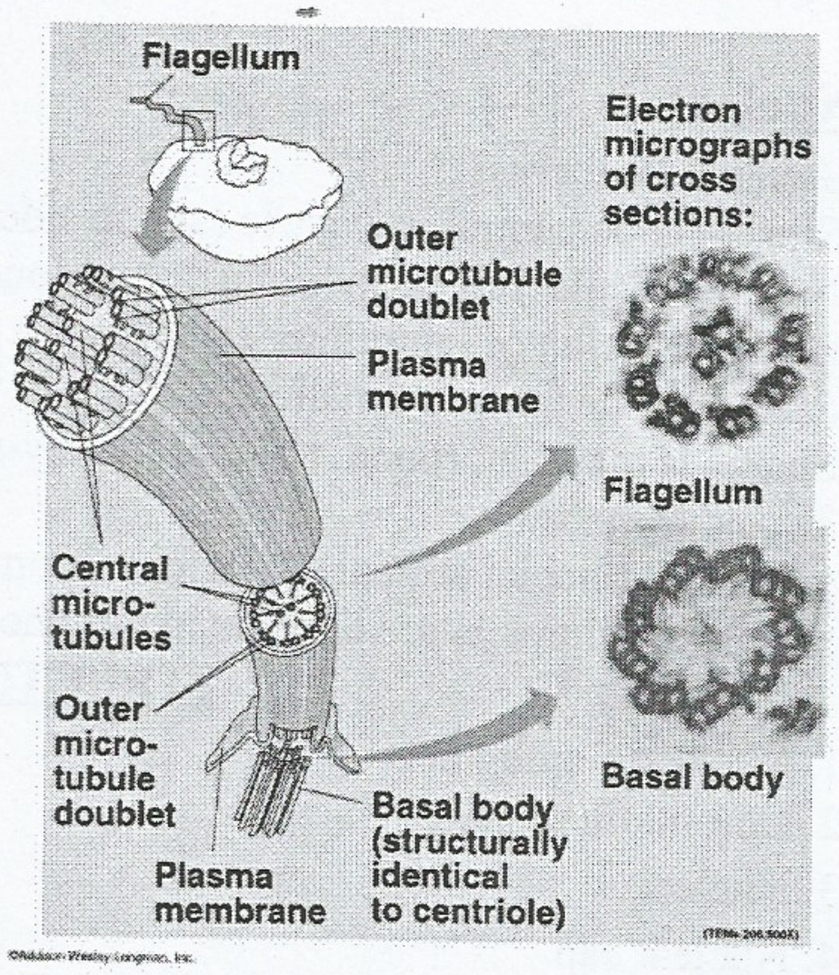
Short rods located in special region of cytoplasm adjacent to the nucleus called centrosome.
Ocular in pairs(diplosome), long axes are at right angles to each other.
Self-duplicate in S period and move to opposite sides during mitosis.
Hollow cylinder 150nm in diameter and 300-500nm in length in EM.
Composed of 9 sets of three microtubules, triplets, in the characteristic pinwheel arrangement.



Pericentriolar bodies represent nucleation centers for microtubule formation.

Functions: 1- Initiation of cell division and spindle formation.

2- Formation of cilia and flagella.



Cilia:

L/M: Appear as hair-like striations on the free surface of the cells.

E/M: Each cilium is formed of shaft, basal body and rootlets:

— The shaft is formed of 2 singlets in the centre of 9 doublets, all surrounded by cell membrane.

— The basal body, has the same structure as centriole (9 triplets).

— The rootiets microtubules extend from basal body to fix the cilium in the cytoplasm

Function: Help movement of particles or fluids in one direction, e.g. in respiratory tract.

Filaments:

Microfilaments

Types of Cytoplasmic filaments: There are three categories of Cytoplasmic filaments:

I- Actin filaments (microfilaments):

- They are the smallest in size, 7nm in diameter.
- They consist mainly of a protein called actin.
- They are present mainly in the striated muscle cells myocytcs) as contractile element.
- They play an important role in cell division as they form the contractile ring during the telophase.
- They are present also in the Core of microvilli, terminal web and blood platelets.

II- Thick or Myosin filaments:

- They are thicker than actin filaments, 15 nrn in diameter
- They are formed of a protein, myosin.
- They are present mainly in the striated muscle cells as a contractile element, in the form of thick filaments.
- They are present also in rnicrovilli, terminal web and blood platelets

Actin and myosin filaments may he called myofilaments.

III- Intermediate filaments

They are 10 nm in diameter, intermediate in size between thin(actin) and thick filaments(myosin) .

They include the following filaments: -

I- Tonofilaments (Keratin filaments) of the epithelium of skin.

ii- Neurofilaments of nerve cells.

iii- Desmin filaments of muscles specially smooth muscle.

iv- Vementin filaments of mesenchyrnal cells as fibroblasts.

Cytoplasmic inclusion

Usually transitory components of cytoplasm, mainly of accumulated metabolites or deposits of varied nature.

1- Glycogen in the liver cells, usually granular, electron-dense particle.

2- Lipid droplets in adipose tissue, adrenal cortex and liver cell.

3- Protein in glandular cells as secretory granules.

4- Pigment: endogenous: melanin in epidermal and retinal cells lipofuscin(pigment of aging).exogenous: e.g.: dust, tattoo marks, carotenes

Nucleus

General Features:

Site: May be central, eccentric (at one side) or peripheral.

Number: Cells may be mononucleated (one nucleus), binucleated (2 nuclei, as in liver cells) or multinucleated (many nuclei, as in osteoclasts).

Size: May be large or small.

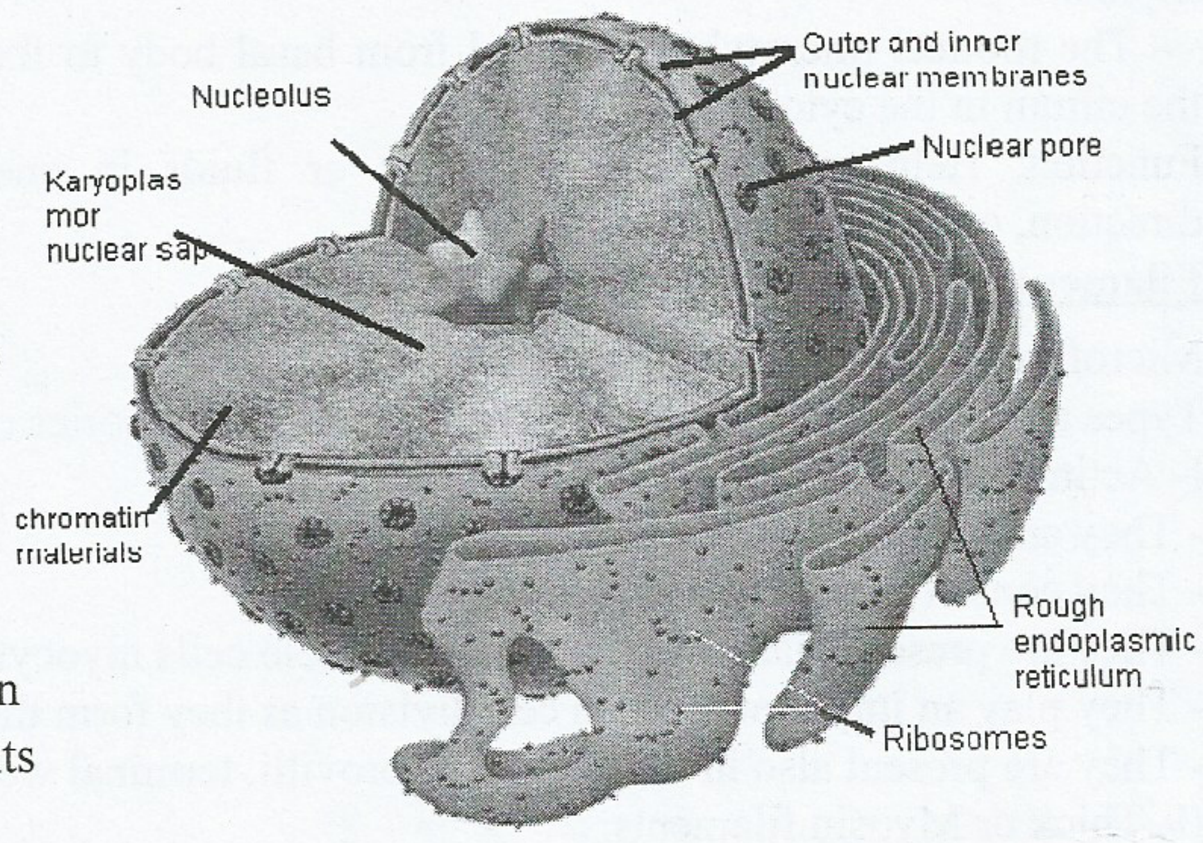
Shape: It may flat, round, oval, or kidney-shaped. It may be lobulated, as inneutrophils.

Types: May be:

- Vesicular nuclei are pale with few chromatin, as in nerve cells.
- Condensed nuclei are dark, as in lymphocytes.

STRUCTURE OF THE NUCLEUS

1. Nuclear membrane.
2. Chromatin material
3. Nucleolus
4. Nuclear sap



1- Nuclear Membrane (Envelope)

LM: Appears as a dark blue line.

EM: It appears as a double walled membrane with many pores.

a) Outer membranous layer: continuous with endoplasmic reticulum, even with ribosomes on its surface.

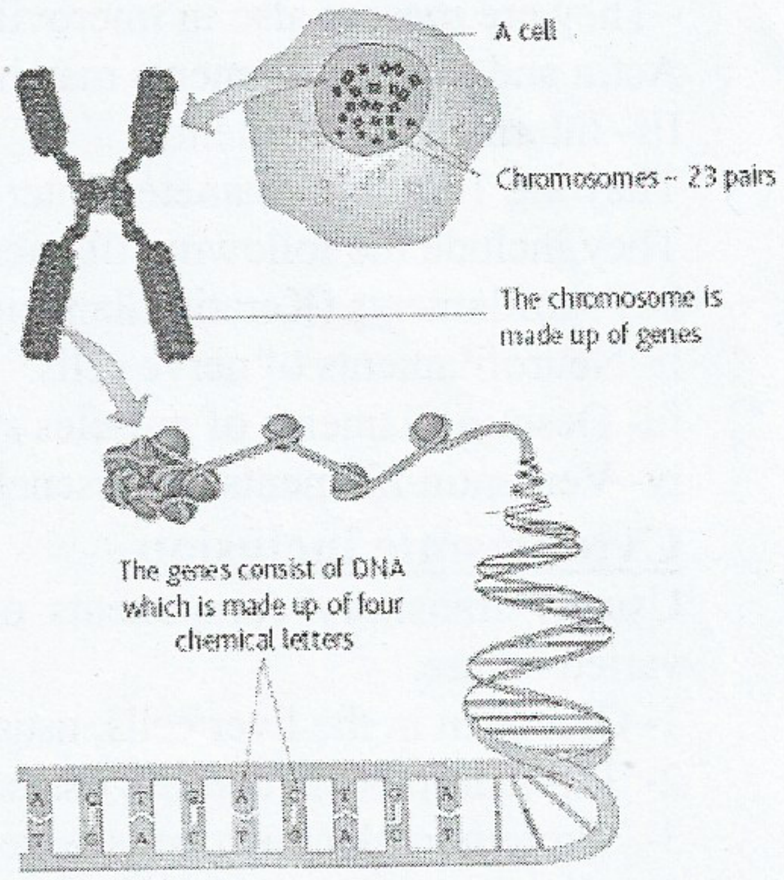
b) Inner membranous layer: with chromatin granules (peripheral chromatin), attached to its inner surface.

c) Nuclear pores, are circular openings in the nuclear membrane. The inner and outer membranes fuse together at the edge of each pore and the opening is covered by a diaphragm. At site of each pore, chromatin material (peripheral chromatin) is deficient. Pores provide communication between nucleus and cytoplasm.

2. Chromatin Material

_ Chromatin material is formed mainly of DNA, which contains the code of genetic information.

LM: Chromatin appears as **basophilic granules**, which may be coarse or fine.



E/M:

_ **Euochromatin** (extended or active). It appears pale and formed of thin uncoiled threads.

_ **Heterochromatin** (condensed or inactive). It is dark and distributed as:

a) Peripheral chromatin, attached to the inner wall of the nuclear membrane.

b) Nucleolus-associated chromatin, around nucleolus.

C) Chromatin islands, scattered in the nucleus.